

GenePrint®

PowerPlex™ 16 System

Technical Manual No. D012

INSTRUCTIONS FOR USE OF PRODUCTS DC6530, DC6531 AND DG2850.

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I. Description

STR^(a) (short tandem repeat) loci consist of short, repetitive sequence elements 3 to 7 base pairs in length (1–4). These repeats are well distributed throughout the human genome and are a rich source of highly polymorphic markers, which may be detected using the polymerase chain reaction (5–8). Alleles of STR loci are differentiated by the number of copies of the repeat sequence contained within the amplified region and are distinguished from one another using radioactive, silver stain or fluorescence detection following electrophoretic separation.

The *GenePrint*[®] PowerPlex[™] 16 System^(a,b) allows the coamplification and three-color detection of sixteen loci (fifteen STR loci and Amelogenin). The *GenePrint*[®] PowerPlex[™] 16 System contains the loci Penta E, D18S51, D21S11, TH01, D3S1358, FGA, TPOX, D8S1179, vWA, Amelogenin, Penta D, CSF1PO, D16S539, D7S820, D13S317 and D5S818. In the *GenePrint*[®] PowerPlex[™] 16 System, one primer specific for Penta E, D18S51, D21S11, TH01 and D3S1358 is labeled with fluorescein (FL); one primer specific for FGA, TPOX, D8S1179, vWA and Amelogenin is labeled with carboxy-tetramethylrhodamine (TMR); and one primer specific for Penta D, CSF1PO, D16S539, D7S820, D13S317 and D5S818 is labeled with 6-carboxy-4',5'-dichloro-2',7'-dimethoxy-fluorescein (JOE). All sixteen loci are amplified simultaneously in a single tube and analyzed in a single injection or gel lane.

The *GenePrint*[®] PowerPlex[™] 16 System is designed specifically for use with the ABI PRISM[®] 310 Genetic Analyzer and is compatible with the ABI PRISM[®] 377 DNA Sequencer.

The *GenePrint*[®] PowerPlex[™] 16 System provides all of the materials necessary for amplification of STR regions of purified genomic DNA with the exception of AmpliTaq Gold[®] DNA polymerase. This manual contains separate protocols for use of the *GenePrint*[®] PowerPlex[™] 16 System with the Perkin-Elmer Model 480 and GeneAmp[®] PCR System 9600, 9700 and 2400 thermal cyclers, in addition to protocols for separation of amplified products and detection of separated material. Protocols for operation of the fluorescence-detecting instrumentation should be obtained from the instrument manufacturer.

For information on Promega's other fluorescent STR systems, refer to the *GenePrint*[®] Fluorescent STR Systems^(a) Technical Manual #TMD006, *GenePrint*[®] PowerPlex[™] 1.1 Fluorescent STR System^(a,b) Technical Manual #TMD008, the *GenePrint*[®] PowerPlex[™] 1.2 System^(a,b) Technical Manual #TMD009 and the *GenePrint*[®] PowerPlex[™] 2.1 STR System^(a) Technical Manual #TMD011. For detection of amplified STR fragments using silver staining (9), refer to the *GenePrint*[®] STR Systems^(a) Technical Manual #TMD004. These Technical Manuals and additional product information are available on the Internet at: www.promega.com or upon request from Promega.

II. STR Typing

A. Advantages of STR Typing

STR typing is more tolerant of the use of degraded DNA templates than other typing methods because the amplification products are less than 500bp long, much smaller than the material detected using AMP-FLP (10) or VNTR (11) analysis. STR typing is also amenable to a variety of rapid DNA purification techniques, which are compatible with PCR but do not provide enough DNA of appropriate quality for Southern blot-based analyses.

GenePrint[®] STR products are generally of discrete and separable lengths. This allows the construction of allelic ladders containing fragments of the same lengths as several or all known alleles for each locus. Visual or instrument-based comparison between the allelic ladder and amplified samples of the same locus allows rapid and precise assignment of alleles. Results obtained using the *GenePrint*[®] PowerPlex[™] 16 System can be recorded in a digitized format, allowing direct comparison with stored databases. Population analyses do not require the use of arbitrarily defined fixed bins for population data (12).

B. Advantages of Using the Loci in the *GenePrint*[®] PowerPlex[™] 16 System

The loci included in the *GenePrint*[®] PowerPlex[™] 16 System (Tables 1 and 2) have been selected because they satisfy the needs of several major standardization bodies throughout the world. The European police network, called INTERPOL, has established a set of four STR loci (FGA, D21S11, TH01 and vWA) as a pan-European standard. The European Network of Forensic Science Institutes (ENFSI) has recommended seven STR loci (FGA, D21S11, TH01, vWA, D8S1179, D18S51 and D3S1358), and GITAD (Grupo Iberoamericano de Trabajo en Análisis de DNA) has recommended six loci (CSF1PO, TH01, TPOX, D16S539, D7S820 and D13S317). The loci amplified in the *GenePrint*[®] PowerPlex[™] 16 System include all of these standard loci.

The United States Federal Bureau of Investigation (FBI) has selected 13 STR core loci to be typed prior to searching or including (submitting) samples in the CODIS (COmbined DNA Index System), the U.S. national database of convicted offender profiles. The *GenePrint*[®] PowerPlex[™] 16 System amplifies all CODIS core loci in a single reaction.

The *GenePrint*[®] PowerPlex[™] 16 System also contains two new low-stutter, highly polymorphic pentanucleotide repeat loci, Penta E and Penta D. These additional loci add significantly to the discrimination power of the system, making the *GenePrint*[®] PowerPlex[™] 16 System a single amplification system with a power of exclusion sufficient to resolve paternity disputes definitively. In addition, the extremely low stutter seen with Penta E and Penta D, makes them ideal loci for evaluation of DNA mixtures often encountered in forensic casework.

Finally, the Amelogenin locus is included in the *GenePrint*[®] PowerPlex[™] 16 System to allow gender identification of each sample. Table 3 lists the *GenePrint*[®] PowerPlex[™] 16 System alleles revealed in commonly available standard DNA templates.

We have carefully selected STR loci and primers to avoid or minimize artifacts, including those associated with *Taq* DNA polymerase, such as repeat slippage and terminal nucleotide addition. Repeat slippage (13,14), sometimes called “n – 4 bands,” “stutter” or “shadow bands,” is due to the loss of a repeat unit during DNA amplification, somatic variation within the DNA in sample material, or both. The amount of this artifact observed is dependent primarily on the locus and the DNA sequence being replicated.

Terminal nucleotide addition (15,16) occurs when *Taq* DNA polymerase adds a nucleotide, generally adenine, to the 3' ends of amplified DNA fragments in a template-independent manner. The efficiency with which this occurs varies with different primer sequences. Thus, an artifact band one base shorter than expected (i.e., missing the terminal addition) is sometimes seen. We have modified primer sequences and added a final extension step of 60°C for 30 minutes (17) to the amplification protocol to provide conditions for essentially full terminal nucleotide addition when recommended amounts of DNA template are used.

The presence of microvariant alleles (alleles differing from one another by lengths other than the repeat length) complicates interpretation and assignment of alleles. There appears to be a correlation between a high degree of polymorphism, a tendency for microvariants and increased mutation rate (18,19). Thus, the FGA and D21S11 display numerous, relatively common microvariants. For reasons yet unknown, the highly polymorphic Penta E locus does not display frequent microvariants (Table 2).

Table 1. The GenePrint® PowerPlex™ 16 System Locus-Specific Information.

STR Locus	Label	Chromosomal Location	GenBank® Locus and Locus Definition	Repeat Sequence ¹ 5'→3'
Penta E	FL	15q	NA	AAAGA
D18S51	FL	18q21.3	HUMUT574	AGAA (20)
D21S11	FL	21q11–21q21	HUMD21LOC	TCTA Complex (20)
TH01	FL	11p15.5	HUMTH01, Human tyrosine hydroxylase gene	AATG (20)
D3S1358	FL	3p	NA	TCTA Complex
FGA	TMR	4q28	HUMFIBRA, Human fibrinogen alpha chain gene	TTTC Complex (20)
TPOX	TMR	2p23–2pter	HUMTPOX, Human thyroid peroxidase gene	AATG
D8S1179	TMR	8q	NA	TCTA Complex (20)
vWA	TMR	12p12–pter	HUMVWFA31, Human von Willebrand factor gene	TCTA Complex (20)
Amelogenin	TMR	Xp22.1–22.3 and Y	HUMAMEL, Human Y chromosomal gene for Amelogenin-like protein	NA
Penta D	JOE	21q	NA	AAAGA
CSF1PO	JOE	5q33.3-34	HUMCSF1PO, Human c-fms proto-oncogene for CSF-1 receptor gene	AGAT
D16S539	JOE	16q24–qter	NA	GATA
D7S820	JOE	7q11.21–22	NA	GATA
D13S317	JOE	13q22–q31	NA	TATC
D5S818	JOE	5q23.3–32	NA	AGAT

¹The August 1997 report (21,22) of the DNA Commission of the International Society for Forensic Haemogenetics (ISFH) states, “1) for STR loci within coding genes, the coding strand shall be used and the repeat sequence motif defined using the first possible 5' nucleotide of a repeat motif; and 2) for STR loci not associated with a coding gene, the first database entry or original literature description shall be used.”

TMR = carboxy-tetramethylrhodamine

FL = fluorescein

JOE = 6-carboxy-4',5'-dichloro-2',7'-dimethoxyfluorescein

NA = Not applicable

Note: Amelogenin is not an STR but displays a 106-base, X-specific band and a 112-base, Y-specific band. 9947A DNA (female) displays only the 106-base, X-specific band.

Table 2. The GenePrint® PowerPlex™ 16 System Allelic Ladder Information.

STR Locus	Label	Size Range of Allelic Ladder Components ^{1,2} (bases)	Repeat Numbers of Allelic Ladder Components	Repeat Numbers of Alleles Not Present in Allelic Ladder ³
Penta E	FL	379–474	5–24	20.3
D18S51	FL	290–366	8–10, 10.2, 11–13, 13.2, 14–27	
D21S11	FL	203–259	24, 24.2, 25, 25.2, 26–28, 28.2, 29, 29.2, 30, 30.2, 31, 31.2, 32, 32.2, 33, 33.2, 34, 34.2, 35, 35.2, 36–38	
TH01	FL	156–195	4–9, 9.3, 10–11, 13.3	
D3S1358	FL	115–147	12–20	
FGA	TMR	322–444	16–18, 18.2, 19, 19.2, 20, 20.2, 21, 21.2, 22, 22.2, 23, 23.2, 24, 24.2, 25, 25.2, 26–30, 31.2, 43.2, 44.2, 45.2, 46.2	
TPOX	TMR	262–290	6–13	
D8S1179	TMR	203–247	7–18	
vWA	TMR	123–171	10–22	
Amelogenin	TMR	106, 112	X, Y	
Penta D	JOE	376–441	2.2, 3.2, 5, 7–17	
CSF1PO	JOE	321–357	6–15	
D16S539	JOE	264–304	5, 8–15	
D7S820	JOE	215–247	6–14	
D13S317	JOE	169–201	7–15	
D5S818	JOE	119–155	7–16	

¹Lengths of each allele in the allelic ladders have been confirmed by sequence analyses.

²When using an internal lane standard such as the Internal Lane Standard 600, calculated sizes of allelic ladder components may differ from those listed. This occurs because different sequences in allelic ladder and ILS components may cause differences in migration. The dye label also affects migration of alleles.

³The alleles listed are those with a frequency of >1/1000.

Note: Amelogenin is not an STR but displays a 106-base, X-specific band and a 112-base, Y-specific band.

Table 3. The GenePrint® PowerPlex™ 16 System Allele Determinations in Commonly Available Standard DNA Templates.

STR Locus	Standard DNA Templates		
	K562	9947A	9948
Penta E	14, 5	13, 12	11, 11
D18S51	16, 15	19, 15	18, 15
D21S11	31, 30, 29	30, 30	30, 29
TH01	9.3, 9.3	9.3, 8	9.3, 6
D3S1358	16, 16	15, 14	17, 15
FGA	24, 21	24, 23	26, 24
TPOX	9, 8	8, 8	9, 8
D8S1179	12, 12	13, 13	13, 12
vWA	16, 16	18, 17	17, 17
Amelogenin	X, X	X, X	X, Y
Penta D	13, 9	12, 12	12, 8
CSF1PO	10, 9	12, 10	12, 11, 10
D16S539	12, 11	12, 11	11, 11
D7S820	11, 9	11, 10	11, 11
D13S317	8, 8	11, 11	11, 11
D5S818	12, 11	11, 11	13, 11

Note: Information on strains 9947A, 9948 and K562 is available online at: locus.umdj.edu/nigms. Strain K562 is available from the American Type Culture Collection, www.atcc.org, (Manassas, VA).

C. Power of Discrimination

The fifteen STR loci amplified with the *GenePrint*[®] PowerPlex[™] 16 System provide powerful discrimination. Population statistics for these loci and their various multiplex combinations are being developed as part of a collaboration with The Bode Technology Group (Springfield, VA). Generation of these data includes analysis of over two hundred individuals from each of the three major racial and ethnic groups in the United States. Preliminary data has been used to generate the population statistics displayed in Tables 4–6. For additional population data for STR loci, see references 23–28.

Table 4 shows the matching probability (29) for the PowerPlex[™] 1.2 and 16 Systems in various populations. The matching probability of the *GenePrint*[®] PowerPlex[™] 16 System ranges from 1 in 1.83×10^{17} for Caucasian-Americans to 1 in 1.42×10^{18} for African-Americans.

A measure of discrimination often used in paternity analyses is the paternity index (PI), a means for presenting the genetic odds in favor of paternity given the genotypes for the mother, child and alleged father (30). The typical Paternity Indices for the PowerPlex[™] 1.2 and 16 Systems are shown in Table 5. The *GenePrint*[®] PowerPlex[™] 16 System provides typical paternity indices exceeding 1,000,000 in each population group.

An alternative calculation used in paternity analyses is the power of exclusion (30). This value, calculated for the *GenePrint*[®] PowerPlex[™] 16 System, exceeds 0.999998 in all populations tested (Table 6).

Table 4. Matching Probabilities of the *GenePrint*[®] PowerPlex[™] 1.2 and 16 Systems in Various Populations.

STR System	Matching Probability			
	African-American	Caucasian-American	Hispanic-American	Asian-American
PowerPlex [™] 1.2 System (8 STR loci)	1 in 2.74×10^8	1 in 1.14×10^8	1 in 1.45×10^8	1 in 1.32×10^8
PowerPlex [™] 16 System (15 STR loci)	1 in 1.42×10^{18}	1 in 1.83×10^{17}	1 in 2.94×10^{17}	1 in 3.74×10^{17}

Table 5. Typical Paternity Indices of the *GenePrint*[®] PowerPlex[™] 1.2 and 16 Systems in Various Populations.

STR System	Typical Paternity Index			
	African-American	Caucasian-American	Hispanic-American	Asian-American
PowerPlex [™] 1.2 System	498	260	319	471
PowerPlex [™] 16 System	2,510,000	1,520,000	5,220,000	4,110,000

Table 6. Power of Exclusion of the *GenePrint*[®] PowerPlex[™] 1.2 and 16 Systems in Various Populations.

STR System	Power of Exclusion			
	African-American	Caucasian-American	Hispanic-American	Asian-American
PowerPlex [™] 1.2 System	0.9982125	0.9968853	0.9973337	0.9981793
PowerPlex [™] 16 System	0.9999996	0.9999994	0.9999983	0.9999998

D. The Internal Lane Standard 600

The Internal Lane Standard (ILS) 600 contains 22 DNA fragments of 60, 80, 100, 120, 140, 160, 180, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, 550 and 600 bases in length. Each fragment is labeled with carboxy-X-rhodamine (CXR) and may be detected separately (as a fourth color) in the presence of PowerPlex™ 16 amplified material using the ABI PRISM® 310 Genetic Analyzer or the ABI PRISM® 377 DNA sequencer. The ILS 600 is designed for use in each gel lane or capillary electrophoresis (CE) injection to increase precision in analyses when using the *GenePrint*® PowerPlex™ 16 System. A protocol for preparation and use of this internal lane standard is provided in Sections VI.C. and VII.E.

E. Warnings and Precautions

The application of PCR-based typing for forensic or paternity casework requires validation studies and quality control measures that are not contained in this manual (31,32). The quality of the purified DNA sample, as well as small changes in buffers, ionic strength, primer concentrations, choice of thermal cycler and thermal cycling conditions can affect the success of a PCR reaction. We suggest strict adherence to recommended procedures for amplification, as well as for denaturing gel electrophoresis and fluorescence detection.

PCR-based STR analysis is subject to contamination by very small amounts of nontemplate human DNA. Extreme care should be taken to avoid cross-contamination when preparing sample DNA, handling primer pairs, setting up amplification reactions and analyzing amplification products. Reagents and materials used prior to amplification (Gold ST★R 10X Buffer and PowerPlex™ 16 10X Primer Pair Mix) are provided in a separate box and should be stored separately from those used following amplification (PowerPlex™ 16 Allelic Ladder Mix, Internal Lane Standard 600 and Blue Dextran Loading Solution). Always include a negative control reaction (i.e., no template) to ensure reagent purity. We highly recommend the use of gloves and aerosol-resistant pipette tips (e.g., ART® tips, Section XI.B).

Some of the reagents used in the analysis of STR products are potentially hazardous and should be handled accordingly. Table 7 describes the potential hazards associated with such reagents.

Table 7. Hazardous Reagents.

Reagents for ABI PRISM® 310 Genetic Analyzer	Hazard
formamide	irritant, teratogen
Reagents for ABI PRISM® 377 DNA Sequencer	Hazard
acrylamide (Long Ranger™ Gel Solution)	suspected carcinogen, toxic
ammonium persulfate	oxidizer, corrosive
formamide (contained in the Blue Dextran Loading Solution)	irritant, teratogen
TEMED	corrosive, flammable
urea	irritant

III. Product Components

Product	Size	Cat.#
<i>GenePrint</i> [®] PowerPlex [™] 16 System	100 reactions	DC6531
	400 reactions	DC6530

DC6531 contains sufficient reagents for 100 reactions of 25µl each. Includes:

Preamplification Components Box (Yellow Label)

- 1 × 300µl Gold ST★R 10X Buffer
- 1 × 250µl PowerPlex[™] 16 10X Primer Pair Mix
- 25µl 9947A DNA (10ng/µl)

Postamplification Components Box (Magenta Label)

- 1 × 25µl PowerPlex[™] 16 Allelic Ladder Mix
- 1 × 150µl Internal Lane Standard (ILS) 600
- 1 × 1ml Blue Dextran Loading Solution
- 1 Technical Manual

Storage Conditions: Store all components at –20°C. The PowerPlex[™] 16 10X Primer Pair Mix, PowerPlex[™] 16 Allelic Ladder Mix and the Internal Lane Standard 600 are light sensitive and must be stored in the dark. We strongly recommend that preamplification and postamplification reagents be stored and used separately with different pipettes, tube racks, etc.

Product	Cat.#
<i>GenePrint</i> [®] PowerTyper [™] 16 Macro	DG2850

The PowerTyper[™] 16 Macro for use with Genotyper[®] 2.0 or 2.5 is available (free of charge) upon request from Promega.

Matrix standards are required for initial set-up of the color separation matrix. The *GenePrint*[®] Matrix FL-JOE-TMR-CXR is sold separately (see Section XI.B).

Additional *GenePrint*[®] Fluorescent STR Multiplex product information, together with ordering information for accessory components and related products, is available on the Internet at: www.promega.com or upon request from Promega.



The Allelic Ladder Mix is provided in a separate, sealed bag for shipping. This component should be moved to the postamplification box after opening.



Please see Related Products (Section XI.B) for information on the *GenePrint*[®] Matrix FL-JOE-TMR-CXR (Cat.# DG2860) and Internal Lane Standard 600 (Cat.# DG2611).

IV. DNA Extraction Methods

We have evaluated a number of DNA extraction methods to prepare DNA for amplification. The SV Total RNA Isolation System^(c) (Cat.# Z3100) can be used for rapid isolation of genomic DNA in as little as 15–30 minutes, using a modification of the standard protocol. For more information on this protocol modification, see reference 33. Promega's ReadyAmp[™] Genomic DNA Purification System (Cat.# A7710) provides a simple, effective and safe approach to isolate genomic DNA from whole blood or bloodstains for amplification analysis. The Wizard[®] Genomic DNA Purification Kit (Cat.# A1120) may be used to isolate double-stranded DNA from whole blood rapidly and simply without the use of organic reagents. The DNA isolated using the Wizard[®] kit is suitable for PCR and Southern hybridization applications. Additional methods are detailed in the *GenePrint*[®] *Fluorescent STR Systems Technical Manual* #TMD006. This Technical Manual is available on the Internet at: www.promega.com or upon request from Promega.

V. Protocols for DNA Amplification Using the *GenePrint*[®] PowerPlex[™] 16 System

Materials to Be Supplied by the User

- Thermal Cycler Model 480 or GeneAmp[®] System 9600, 9700 or 2400 (PE Biosystems)
- microcentrifuge
- 0.5ml or 0.2ml (thin-walled) microcentrifuge tubes (PE Biosystems)
- 1.5ml amber-colored microcentrifuge tubes (Fisher Cat.# 05-402-26)
- aerosol-resistant pipette tips (see Section XI.B)
- AmpliTaq Gold[®] DNA polymerase (PE Biosystems)
- Nuclease-Free Water (Cat.# P1193)
- mineral oil (Cat.# DY1151, for use with the Thermal Cycler Model 480)

We routinely amplify 0.5–1ng of template DNA in a 25µl reaction volume using the protocols detailed below. If more template is used, expect to see high peaks in the smaller loci and relatively lower peak heights in the larger loci. The *GenePrint*[®] PowerPlex[™] 16 System is optimized for the GeneAmp[®] PCR System 9600 thermal cycler. Amplification protocols for the GeneAmp[®] PCR Systems 9700 and 2400 thermal cyclers and the Perkin-Elmer Model 480 thermal cycler are provided.

A. Amplification Set-Up

Note: The use of gloves and aerosol-resistant pipette tips is **highly** recommended to prevent cross-contamination. Keep all preamplification and post-amplification reagents in separate rooms. Prepare amplification reactions in a room dedicated for reaction setup. Use equipment and supplies dedicated for amplification setup.

1. Thaw the Gold ST★R 10X Buffer and PowerPlex[™] 16 10X Primer Pair Mix.

Note: It is very important to mix these reagents by vortexing for 5–10 seconds before each use. A precipitate may form in the Gold ST★R Buffer. If this occurs, warm the solution briefly at 37°C, then vortex until it is in solution.

2. Determine the number of reactions to be set up. This should include positive and negative control reactions. Add 1 or 2 reactions to this number to compensate for pipetting error. While this approach does waste a small amount of each reagent, it ensures that you will have enough PCR master mix for all samples. It also ensures that each reaction contains the same master mix.
3. Place one clean, 0.2ml or 0.5ml microcentrifuge tube for each reaction into a rack and label appropriately.
4. Table 8 shows the component volumes per sample when using a DNA template volume of 2.5µl in a 25µl reaction volume. If a larger volume of template is required, the water volume should be adjusted accordingly.

Table 8. Amplification Mix for the *GenePrint*[®] PowerPlex[™] 16 System.

PCR Master Mix Component	Volume Per Sample
Nuclease-Free Water	16.7µl
Gold ST★R 10X Buffer	2.5µl
PowerPlex [™] 16 10X Primer Pair Mix	2.5µl
AmpliTaq Gold [®] DNA polymerase ¹	0.8µl (4u)
Master Mix Volume	22.5µl
Template DNA (to be added)	2.5µl
Total reaction volume	25µl

¹Assumes the AmpliTaq Gold[®] DNA polymerase is at 5u/µl. If the enzyme concentration is different, the volume of enzyme used must be adjusted accordingly.



Meticulous care must be taken to ensure successful amplification. A guide to amplification troubleshooting is provided in Section IX.A.

Note: If using the GeneAmp[®] PCR System 9600, 9700 or 2400 thermal cyclers, use 0.2ml thin-walled MicroAmp[®] reaction tubes. For the Perkin-Elmer Model 480, the standard 0.5ml GeneAmp[®] reaction tubes are recommended.

- Use Table 9 to calculate the required amount of each component of the PCR master mix. Multiply the volume (μl) per sample by the total number of reactions (from Step 2) to obtain the final master mix volume (μl).

Table 9. Master Mix for the GenePrint® PowerPlex™ 16 System.

PCR Master Mix Component	Volume Per Sample	\times	Number of Reactions	Final Volume (μl)
Nuclease-Free Water	μl			
Gold ST★R 10X Buffer	2.5 μl			
PowerPlex™ 16 10X Primer Pair Mix	2.5 μl			
AmpliTaq Gold® DNA polymerase ¹	0.8 μl (4u)			
master mix volume ²	μl			

Per Tube

template DNA volume (to be added)	μl
total reaction volume	25 μl

¹Assumes the AmpliTaq Gold® DNA polymerase is at 5u/ μl . If the enzyme concentration is different, the volume of enzyme used must be adjusted accordingly.

²The Master Mix Volume added to the Template DNA Volume should equal 25 μl .

Note: If the template DNA is stored in TE buffer, the volume of the DNA sample added should not exceed 20% of the final reaction volume. PCR amplification efficiency and quality can be greatly altered by changes in pH (due to added Tris-HCl), available magnesium concentration (due to chelation by EDTA) or other PCR inhibitors, which may be present at low concentrations depending on the source of the template DNA and the extraction procedure used.

- In the order listed in Table 9, add the final volume of each reagent into a sterile, 1.5ml amber-colored tube. Mix gently.
- Pipet PCR master mix into each reaction tube (Table 9).
- Pipet the template DNA (0.5–1ng) of each sample into the respective tube containing PCR master mix.
- For the positive amplification control, dilute the 9947A DNA sample to 1ng in the template DNA volume. Pipet 1ng of the diluted DNA into a microcentrifuge tube containing the PCR master mix.
- For the negative amplification control, pipet Nuclease-Free Water (instead of template DNA) into a microcentrifuge reaction tube containing the PCR master mix.
- If using the GeneAmp® PCR System 9600, 9700 or 2400 thermal cycler and MicroAmp® reaction tubes, no addition of mineral oil to the reaction tubes is required. However, if using the Model 480 thermal cycler and GeneAmp® reaction tubes, add 1 drop of mineral oil to each tube before closing.

Note: Allow the mineral oil to flow down the side of the tube and form an overlay to limit sample loss or cross-contamination due to splattering.



Amplification

of >2ng of DNA template results in an imbalance in peak heights from locus to locus. The smaller loci show greater amplification yield than the larger loci. Reducing the number of cycles in the amplification program by 2 to 4 cycles (i.e., 10/20 or 10/18 cycling) can improve locus-to-locus balance.

B. Amplification Thermal Cycling

1. Assemble the tubes in a thermal cycler.
2. Select and run a recommended protocol. The preferred protocols for use with the GeneAmp® PCR system 9600, the GeneAmp® PCR system 9700, the GeneAmp® PCR system 2400 and the Perkin-Elmer model 480 thermal cyclers are provided below.

Protocol for the Perkin-Elmer GeneAmp® PCR System 9600 Thermal Cycler

Cycling profile: 95°C for 11 minutes, then:
96°C for 1 minute, then:
94°C for 30 seconds
ramp 68 seconds to 60°C, hold for 30 seconds
ramp 50 seconds to 70°C, hold for 45 seconds
for **10 cycles**, then:
90°C for 30 seconds
ramp 60 seconds to 60°C, hold for 30 seconds
ramp 50 seconds to 70°C, hold for 45 seconds
for **22 cycles**, then:
60°C for 30 minutes
4°C soak

Protocol for the Perkin-Elmer GeneAmp® PCR System 9700 Thermal Cycler

Cycling profile: 95°C for 11 minutes, then:
96°C for 1 minute, then:
ramp 100% to
94°C for 30 seconds,
ramp 29% to
60°C for 30 seconds
ramp 23% to
70°C for 45 seconds
for **10 cycles**, then:
ramp 100% to
90°C for 30 seconds
ramp 29% to
60°C for 30 seconds
ramp 23% to
70°C for 45 seconds
for **22 cycles**, then:
60°C for 30 minutes
4°C soak

Protocol for the Perkin-Elmer GeneAmp® PCR System 2400 Thermal Cycler

Cycling profile: 95°C for 11 minutes, then:
96°C for 1 minute, then:
ramp 100% to
94°C for 30 seconds
ramp 100% to
60°C for 30 seconds
ramp 23% to
70°C for 45 seconds
for **10 cycles**, then:
ramp 100% to
90°C for 30 seconds
ramp 100% to
60°C for 30 seconds
ramp 23% to
70°C for 45 seconds
for **22 cycles**, then:
60°C for 30 minutes
4°C soak

Protocol for the Perkin-Elmer Thermal Cycler Model 480

Cycling profile: 95°C for 11 minutes, then:
96°C for 2 minutes, then:
94°C for 1 minute
60°C for 1 minute
70°C for 1.5 minutes
for **10 cycles**, then:
90°C for 1 minute
60°C for 1 minute
70°C for 1.5 minutes
for **22 cycles**, then:
60°C for 30 minutes
4°C soak

3. After completion of the thermal cycling protocol, store the samples at –20°C in a light-protected box.

Note: Storage of amplified samples at 4°C or higher may produce degradation products.

C. Agarose Gel Electrophoresis of Amplification Products (Optional)

Agarose gel electrophoresis can be used to rapidly confirm the success of the amplification reaction prior to performing polyacrylamide gel or capillary electrophoresis.

Materials to Be Supplied by the User

(Solution compositions are provided in Section XI.A.)

- TAE 1X buffer
 - agarose
 - 5X loading solution
 - ethidium bromide solution, 0.5µg/ml
1. Prepare a 2% agarose gel (approximately 150cm²) by adding 2.0g of agarose to 100ml of TAE 1X buffer. Mark the liquid level on the container, then boil or heat in a microwave oven to dissolve the agarose. Add preheated (60°C) deionized water to make up for any volume lost due to evaporation.
 2. Cool the agarose to 55°C before pouring into the gel tray. Be sure that the gel tray is level. Pour the agarose into the tray, insert the gel comb and allow to set for 20–30 minutes.
 3. Prepare the samples by mixing 10µl of each amplified sample with 2.5µl of 5X loading solution.
 4. Prepare 1 liter of TAE 1X buffer for the electrophoresis running buffer.
 5. Place the gel and tray in the electrophoresis gel box. Pour enough running buffer into the tank to cover the gel to a depth of at least 0.65cm. Gently remove the comb.
 6. Load each sample mixed with 5X loading solution (see Step 3, above).
 7. Set the voltage at 5 volts/cm (measured as the distance between the two electrodes). Allow the gel to run for 2 hours.
 8. After electrophoresis, stain the gel in TAE 1X buffer containing 0.5µg/ml ethidium bromide. Gently rock for 20 minutes at room temperature. Remove the ethidium bromide solution and replace with deionized water. Allow the gel to destain for 20 minutes.
 9. Photograph the gel using a UV transilluminator (302nm).

Note: When analyzing the data, do not be alarmed if you see extra bands in addition to the alleles. DNA heteroduplexes can be expected when performing nondenaturing agarose gel electrophoresis. The sole purpose of the agarose gel is to confirm the success of the PCR reaction.

VI. Detection of Amplified Fragments Using the ABI PRISM® 310 Genetic Analyzer

Materials to Be Supplied by the User

(Solution compositions are provided in Section XI.A.)

- dry heating block, water bath, or thermal cycler
- 310 capillaries, 47cm × 50µm (PE Biosystems)
- Performance Optimized Polymer 4 (POP-4; PE Biosystems)
- glass syringe (1ml)
- 10X Genetic Analyzer Buffer with EDTA (PE Biosystems)
- sample tubes and septa (PE Biosystems)
- aerosol-resistant pipette tips (Section XI.B.)
- deionized formamide with conductivity <100µS/cm (Amresco Ultra Pure Grade Cat. #0606)
- *GenePrint*® Matrix FL-JOE-TMR-CXR (Cat.# DG2860)
- ice



The quality of the formamide is critical. Use deionized formamide with a conductivity less than 100µS/cm. Formamide can be aliquoted and frozen at –20°C. Multiple freeze-thaws or long-term storage at 4°C may cause a breakdown of the formamide. Formamide with a conductivity greater than 100µS/cm may contain ions that compete with DNA during injection. This results in lower peak heights and reduced sensitivity. A longer injection time may not increase the signal.

A. Matrix Standardization

The *GenePrint*® Matrix FL-JOE-TMR-CXR (Cat.# DG2860) is required for matrix standardization. For protocols and additional information on matrix standardization, see the *Matrix FL-JOE-TMR-CXR Technical Bulletin #TBD015* (supplied with Cat.# DG2860) available upon request from Promega or online at www.promega.com. Proper generation of a matrix file is critical to evaluate multi-color systems with the ABI PRISM® 310 Genetic Analyzer. A matrix must be generated for each ABI PRISM® 310 Genetic Analyzer.

B. Instrument Preparation

1. Refer to the *ABI PRISM® 310 Genetic Analyzer User's Manual* for instructions on cleaning the pump block, installing the capillary, calibrating the autosampler and adding polymer to the syringe.
2. Open the ABI PRISM® 310 Collection Software.
3. Prepare a GeneScan® sample sheet as described in the *ABI PRISM® 310 Genetic Analyzer User's Manual*. Enter the appropriate sample information in the “sample info” column.

For rows containing the PowerPlex™ 16 Allelic Ladder Mix, insert “Ladder” in the “sample info” column for the blue dye color, yellow dye color and green dye color. This information must be entered to successfully analyze your data using the PowerTyper™ 16 Macro.

Create a new GeneScan® injection list. Select the appropriate sample sheet by using the pull-down menu.



INJECTION TIME

at Step 4 may need to be optimized for individual instruments. Injection times of 2–5 seconds are recommended for 1ng amplification samples.

4. Select the “GS STR POP4 (1ml) A” Module using the pull-down menu. Change the injection time to 3 seconds and the run time to 30 minutes and keep the settings for the remaining parameters as shown below:

Inj. Secs:	3
Inj. kV:	15.0
Run kV:	15.0
Run °C:	60
Run Time:	30

Note: Migration of fragments may vary slightly over the course of a long ABI PRISM® 310 Genetic Analyzer run. This may be due to changes in temperature or changes in the column. When analyzing many samples, injections of the allelic ladder at different times throughout the run can aid in the accurate genotyping of samples.

5. Select the appropriate matrix file (Section VI.A).
6. To analyze the data automatically, select the auto analyze checkbox and the appropriate analysis parameters and size standard. Refer to the *ABI PRISM® 310 Genetic Analyzer User's Manual* for specific information on these options.

C. Sample Preparation

The Internal Lane Standard 600 is included in the *GenePrint® PowerPlex™ 16 System* as the internal lane standard (ILS) for four-color detection and analysis of amplified samples. With this approach, only 3–4 injections of the PowerPlex™ 16 Allelic Ladder Mix are required per 96-sample run. Migration of fragments may vary slightly over the course of an ABI PRISM® 310 Genetic Analyzer run with many samples. This may be due to changes in temperature or changes in the column. Injections of the allelic ladder at different times throughout the run can aid in the accurate genotyping of samples.

1. Prepare a loading cocktail by combining and mixing the Internal Lane Standard 600 (ILS 600) and deionized formamide as follows:
 $[(1.0\mu\text{l ILS 600}) \times (\# \text{ injections})] + [(24.0\mu\text{l deionized formamide}) \times (\# \text{ injections})]$
2. Combine 25.0 μl of the prepared loading cocktail and 1.0 μl of amplified sample.

Note: Instrument detection limits vary; therefore, injection time or the amount of product mixed with loading cocktail may need to be increased or decreased. If the peak heights are too high (i.e., greater than 2,000rfu), the samples can be diluted in Gold ST★R 1X Buffer before mixing with loading cocktail. This may result in uneven allele peak heights across loci. For best results, use less DNA template in the amplification reactions or reduce the number of cycles in the amplification program by 2–4 cycles (i.e., 10/18 or 10/20 cycling). The volume of ILS 600 can also be adjusted.

3. Combine 25.0 μl of the prepared loading cocktail and 1.0 μl of the PowerPlex™ 16 Allelic Ladder Mix.
4. Denature the samples and ladder by heating at 95°C for 3 minutes and immediately chill on ice for 3 minutes. Denature the samples just prior to loading.
5. Assemble the tubes in the appropriate autosampler tray (48- or 96-tube).
6. Place the autosampler tray in the instrument and close the instrument doors.

D. Capillary Electrophoresis and Detection

1. After loading the sample tray and closing the doors, select “Run” to start the capillary electrophoresis system.
2. Monitor the electrophoresis by observing the raw data and status windows.
3. Each sample will take approximately 40 minutes for syringe pumping, sample injection and sample electrophoresis.
4. Analyze the data using the GeneScan® Analysis Software. Open the GeneScan® project.
5. Review the raw data for one or more sample runs. Highlight the sample file name; then under the “sample” menu select “raw data.” Move the cursor so the crosshair is on the baseline to the right of the large primer peak (before the first internal lane standard peak [red]). Use the X-value number shown at the bottom left of the window for the start position in the analysis parameters.
6. The analysis parameters should be:

Analysis Range	Start: defined in step 5 Stop: 10000
Data Processing	Baseline: Checked MultiComponent: Checked Smooth Options: Light*
Peak Detection	Peak Amplitude Thresholds**: B: Y: G: R: Min. Peak Half Width: 3pts
Size Call Range	Min: 60 Max: 600
Size Calling Method	Local Southern Method
Split Peak Correction	None

*Smoothing options should be determined by individual laboratories. Occasionally the TH01 alleles 9.3 and 10 will not be distinguished using heavy smoothing.

**The peak amplitude thresholds are the minimum peak height that the software will call as a peak. Values for the peak amplitude thresholds are usually 50–200rfu and should be determined by individual laboratories.

7. The analysis parameters should be saved in the Analysis Parameters Folder found in the GeneScan® Analysis Folder.
8. Apply the stored analysis parameters file to the samples.
9. Assign a new size standard. Select a sample file and highlight the arrow next to size standard, then select “define new.” Assign the size standard peaks as shown in Panel D of Figure 1, Section VIII.D. Store the size standard in the Size Standards Folder found in the GeneScan® Analysis Folder.

Note: If pull-up from TMR (yellow) is seen in the CXR (red) channel, the peak amplitude threshold can be increased (i.e., 100–200rfu) for the red channel in the analysis parameters. This does not interfere with interpretation, as the CXR (ILS) fragments should be greater than 300rfu.
10. Apply the size standard file to the samples, then analyze the sample files.

Notes:

Amplified sample peak heights of less than 2,000rfu are ideal.

Peak heights >2,000rfu may generate artifact peaks due to instrument saturation (i.e., overloading the sample). If the sample peak heights are not within the linear detection of the instrument, the ratio of stutter peaks to real allele peaks increases and allele designations become difficult to interpret. The balance of the peak heights may also appear less uniform.

There may be variation between instruments regarding their relative fluorescent units detected using the same sample. Furthermore, the different instruments vary in the relative efficiency of color detection affecting the dye color to dye color balance.

11. See Section VIII for Data Analysis.

VII. Detection of Amplified Fragments Using the ABI PRISM® 377 DNA Sequencer

Materials to Be Supplied by the User

(Solution compositions are provided in Section XI.A.)

- dry heating block, water bath or thermal cycler
- Long Ranger™ gel solution (BMA Cat.# 50611) or Long Ranger Singel™ pack for ABI sequencers 377-36cm (BMA Cat.# 50691)
- 10% ammonium persulfate (Cat.# V3131)
- TEMED (Cat.# V3161)
- Urea (Cat.# V3171)
- TBE 10X buffer
- Nalgene® tissue culture filter (0.2 micron)
- aerosol-resistant pipette tips (Section XI.B.)
- gel-loading pipette tips
- 36cm front and rear glass plates
- 36cm gel spacers (0.2mm thick)
- 36-well sharktooth comb or 34-well squaretooth comb (0.2mm thick)
- clamps (e.g., large office binder clamps)
- Liqui-Nox® or other detergent
- GenePrint® Matrix FL-JOE-TMR-CXR (Cat.# DG2860)
- ice

A. Polyacrylamide Gel Preparation

Acrylamide (Long Ranger™ gel solution) is a neurotoxin and suspected cancer agent; avoid inhalation and contact with skin. Read the warning label and take the necessary precautions when handling this substance. Always wear double gloves and safety glasses when working with acrylamide solutions.

The following protocol is for preparation of a 36cm denaturing polyacrylamide gel for use with the ABI PRISM® 377 DNA Sequencer. Low-fluorescence glass plates are recommended and may be obtained from the instrument manufacturer.

1. Thoroughly clean the glass plates with hot water and a 1% Liqui-Nox® solution. Rinse extremely well using deionized water. Allow the glass plates to air-dry in a dust-free environment.
2. Assemble the glass plates by placing 0.2mm side gel spacers between the front and rear glass plates. Hold the plates together using binder clamps (4 clamps on each side). Place the assembly horizontally on a test tube rack or similar support.

- Prepare a 5% Long Ranger™ acrylamide gel (total of 50ml) by adding together the ingredients listed in Table 10. Stir the solution until the urea has dissolved.

Table 10. Preparation of a 5% Long Ranger™ Polyacrylamide Gel.

Component	5% Gel	Final Concentration
Urea	18g	6M
deionized water	26ml	—
10X TBE	5ml	1X
50% Long Ranger™ gel solution	5ml	5%
total volume	50ml	

Note: Long Ranger Singel™ Packs may be used.

- Filter the acrylamide solution through a 0.2 micron filter (e.g., Nalgene® tissue culture filter) and degas for an additional 5 minutes.
- Add 35µl of TEMED and 250µl of fresh 10% ammonium persulfate to the 50ml of acrylamide solution and mix gently.
- Using a disposable 30cc syringe, pour the gel by starting at the well end of the plates and carefully injecting the acrylamide between the horizontal glass plates. Allow the solution to fill the top width of the plates. While maintaining a constant flow of solution, gently tap the glass plates to assist the movement of solution to the bottom of the plates and to prevent the formation of bubbles.
- Insert a 36-well sharktooth comb or 34-well squaretooth comb between the glass plates. Sharktooth combs with 64 or 96 wells may also be used.
- Secure the comb with 3 evenly spaced clamps.
- Keep the remaining acrylamide solution as a polymerization control.
- Allow polymerization to proceed for at least 2 hours. Check the polymerization control to be sure that polymerization has occurred.

B. Matrix Standardization

The *GenePrint*® Matrix FL-JOE-TMR-CXR Kit (Cat.# DG2860) is required for matrix standardization. For protocols and additional information on matrix standardization, see the *GenePrint*® Matrix FL-JOE-TMR-CXR Technical Bulletin #TBD015 (supplied with Cat.# DG2860) available upon request from Promega or at www.promega.com. Proper generation of a matrix file is critical to evaluate multicolor systems with the ABI PRISM® 377 DNA Sequencer. A matrix must be generated for each ABI PRISM® 377 DNA Sequencer.

C. Instrument Preparation

- Open the ABI PRISM® 377 Collection Software.
- Prepare a sample sheet as described in the *GeneScan*® Analysis Software User's Manual. Enter the appropriate sample information in the "sample info" column.

For rows containing the PowerPlex™ 16 Allelic Ladder Mix, insert "Ladder" in the "sample info" column for the blue dye color, yellow dye color and green dye color. This information must be entered to successfully analyze your data using the PowerTyper™ 16 Macro (Cat.# DG2850).

Note: The gel may be stored overnight if a paper towel saturated with deionized water and plastic wrap are placed around the top and bottom to prevent the gel from drying out (crystallization of the urea will destroy the gel).

3. Create a new GeneScan® run and use the following settings:

Plate Check Module:	Plate Check A
PreRun Module:	PR GS 36A-2400
Run Module:	GS 36A-2400
Collect time:	3 hours
Well-to-Read distance:	36cm

4. Select the appropriate sample sheet and comb selection by using the pull-down menus.
5. Select the appropriate gel matrix file (Section VII.B).

D. Gel Prerun

1. Remove the clamps from the polymerized acrylamide gel. If necessary, clean any excess acrylamide from the glass plates with paper towels saturated with deionized water.
2. Shave any excess polyacrylamide away from the comb and remove the comb. If using a sharktooth comb, carefully insert the sharktooth comb teeth into the gel approximately 1–2mm.
3. Position the gel/glass plate unit in the 377 cassette.
4. Secure the cassette in the instrument and perform a plate check as recommended in the *ABI PRISM® 377 DNA Sequencer User's Manual*. If the horizontal line graph is not flat, remove the cassette, clean the plate surface, and repeat the plate check.
5. Add TBE 1X buffer to the top and bottom buffer chambers of the instrument.
6. Using a 30cc syringe filled with buffer, remove any air bubbles from the well area of the gel and place the lid on the upper buffer chamber. Using a syringe with a bent 19-gauge needle, remove any air bubbles from the bottom of the gel.
7. Attach the heating plate, connect the water tubing, attach all electrodes, close the instrument door, and select the "PreRun" button. Allow the gel to prerun for 15–20 minutes or until the gel temperature is at least 40°C. Open the status window to monitor the temperature of the gel.
8. Prepare the sample and allelic ladder samples during the gel prerun.

E. Sample Preparation and Loading

The Internal Lane Standard 600 (ILS 600) is included in the *GenePrint*[®] PowerPlex[™] 16 System for four-color detection and analysis of amplified samples. With this approach, only 2–3 lanes of the PowerPlex[™] 16 Allelic Ladder Mix are required per gel.

1. Prepare a loading cocktail by combining and mixing the ILS 600 and Blue Dextran Loading Solution as follows:
$$[(0.5\mu\text{l ILS 600}) \times (\# \text{ lanes})] + [(1.5\mu\text{l Blue Dextran Loading Solution}) \times (\# \text{ lanes})]$$
2. Combine 2.0 μl of the prepared loading cocktail and 1.0 μl of amplified sample.
Note: Instrument detection limits vary; therefore, the amount of product mixed with loading cocktail may need to be increased or decreased. If the peak heights are too high (i.e., greater than 2,000rfu), the samples can be diluted in Gold ST \star R 1X Buffer before mixing with loading cocktail. This may result in uneven allele peak heights across loci. For best results, use less DNA template in the amplification reactions or reduce the number of cycles in the amplification program by 2–4 cycles (i.e. 10/18 or 10/20 cycling). The volume of ILS 600 can also be adjusted.
3. Combine 2.0 μl of the prepared loading cocktail and 1.0 μl of the PowerPlex[™] 16 Allelic Ladder Mix.
4. Briefly spin the samples in a microcentrifuge to bring the contents to the bottom of the tubes.
5. Denature the samples by heating at 95°C for 2 minutes and immediately chill on ice. Denature the samples just prior to loading the gel. Sample DNA will partially reanneal if denatured hours before loading.
6. After the 15–20 minute prerun, pause the instrument by clicking on the “pause” button. By pausing the prerun, the water will continue to circulate to keep the gel warm during the sample loading.
7. Use a 30cc syringe filled with buffer to flush the urea from the well area.
8. Load 1.5 μl of each denatured sample into the respective wells.
9. Place the lid on the upper buffer chamber and close the instrument door.

F. Gel Electrophoresis and Detection

1. After loading, select “Cancel” to stop the prerun. Make sure that the run time is set at 3 hours, then select “Run” to begin electrophoresis.
2. Monitor the electrophoresis by observing the gel image and status windows.
3. Allow electrophoresis to proceed for 3 hours. The 600-base ILS fragment will have migrated past the laser.
4. Track and extract the gel lanes.
5. Review the gel image. Put the cursor on the gel so that the primer region is excluded. Use the scan value shown at the top of the window for the “Start” value in the analysis parameters.
6. Open the GeneScan[®] project.

 **Step 8** may need to be optimized for individual instruments. Loading volumes of 1.0–2.0 μl are recommended.

7. The recommended analysis parameters are:

Analysis Range	Start: defined in Step 5 Stop: 10000
Data Processing	Baseline: Checked Multi-Component: Checked Smooth Options: Light*
Peak Detection	Peak Amplitude Thresholds**: B: Y: G: R: Min. Peak Half Width: 3pts
Size Call Range	Min: 60 Max: 600
Size Calling Method	Local Southern Method
Split Peak Correction	None

*Smoothing options should be determined by individual laboratories. Occasionally the TH01 alleles 9.3 and 10 will not be distinguished using heavy smoothing.

**The peak amplitude thresholds are the minimum peak height that the software will call as a peak. Values for the peak amplitude thresholds are usually 50–200rfu and should be determined by individual laboratories.

8. The analysis parameters should be saved in the Analysis Parameters Folder found in the GeneScan® Analysis Folder.
9. Apply the stored analysis parameters file to the samples.
10. Assign a new size standard. Select a sample file and highlight the arrow next to size standard, then select “define new.” Assign the size standard peaks shown in Panel D of Figure 1, Section VIII.D. Save the size standard in the Size Standards Folder found in the GeneScan® Analysis Folder.
11. Apply the stored size standard to the sample and then analyze the sample files.

Notes:

Amplified sample peak heights of less than 2,000rfu are ideal.

Peak heights greater than 2,000rfu may generate artifact peaks due to instrument saturation (i.e., overloading the sample). If the sample peak heights are not within the linear detection of the instrument, the ratio of stutter peaks to real allele peaks increases and allele designations become difficult to interpret. The balance of the peak heights may also appear less uniform.

There may be variation between instruments regarding their relative fluorescent units detected using the same sample. Furthermore, the different instruments vary in the relative efficiency of color detection affecting the dye color to dye color balance.

12. See Section VIII for Data Analysis.

G. Reuse of Glass Plates

Separate the glass plates and discard the gel. Clean the glass plates with hot water and a detergent such as 1% Liqui-Nox® detergent. Rinse extremely well with deionized water and allow the plates to air-dry. Do not scrape the plates with abrasive materials during this process.

Soap and oil may build up on plates resulting in gel extrusion or hazy background. Plates may be soaked in 2N HCl for 15 minutes, then rinsed thoroughly.

VIII. Data Analysis

A. PowerTyper™ 16 Macro

To facilitate analysis of the data generated with the *GenePrint*® PowerPlex™ 16 System, we have created a file to allow automatic assignment of genotypes using the ABI Genotyper® Software. After samples have been amplified using the *GenePrint*® PowerPlex™ 16 System, detected using the ABI PRISM® 310 Genetic Analyzer or 377 DNA Sequencer, and analyzed using the GeneScan® Analysis Software, the sample files can be imported into the Genotyper® program and analyzed using the PowerTyper™ 16 Macro (Cat.# DG2850).

The PowerTyper™ 16 Macro is used in conjunction with Genotyper® 2.0 or 2.5 Software. Therefore, Genotyper® 2.0 or 2.5 Software must be installed on your Macintosh® computer.

Be certain the “sample info” column for each lane containing the PowerPlex™ 16 Allelic Ladder Mix contains the word “ladder.” The macro uses the word “ladder” to identify the sample file(s) containing allelic ladder.

Follow the instructions below to determine allele designations for the sixteen loci (Penta E, D18S51, D21S11, TH01, D3S1358, FGA, TPOX, D8S1179, vWA, Amelogenin, Penta D, CSF1PO, D16S539, D7S820, D13S317 and D5S818) contained in the *GenePrint*® PowerPlex™ 16 System. Amplified sample peak heights of less than 2,000rfu are ideal.

B. Analysis

1. Transfer the PowerTyper™ 16 Macro (Cat.# DG2850) from the diskette to a designated location on your computer hard drive.
2. Open the Genotyper® Software and then the PowerTyper™ 16 Macro. For questions regarding the Genotyper® Software, refer to the *Genotyper*® *User's Manual*.
3. Under file, select “import” and import the GeneScan® Project or sample files to be analyzed. Import the blue, yellow, green and red dye colors. (For Genotyper® 2.5 the color selection is found in preferences.)
4. Double-click on the “Check ILS” macro. (The macros are listed at the bottom left corner of the active window.) A plots window will be displayed to show the internal lane standard (i.e., ILS 600) in the red dye color. Scroll down to view and confirm that the internal lane standard fragment sizes are correct. If necessary, reanalyze samples using the GeneScan® Software and redefine the internal lane standard fragments.

Note: On an ABI PRISM® 310 Genetic Analyzer, if the yellow dye color (TMR-labeled loci FGA, TPOX, D8S1179, vWA and Amelogenin) contains peak heights greater than 1,000rfu, bleed-through (generally <150rfu) may be observed in the red dye color. This does not affect interpretation and allele designations of loci in the blue, green and yellow dye colors. If desired, the peak amplitude threshold for the red (ILS 600) channel can be increased (i.e., 150–200rfu) in the analysis parameters to prevent these peaks from being detected.

Note: “Sample info” can be added or modified after importing into PowerTyper™. Highlight the sample, then select “show dye/lanes window” under “views.”

Note: The software uses one ladder sample for determining the allele sizes. The macro uses the **first** ladder sample imported for allele designations.

Note: It may take several minutes to run the “POWER” macro.

5. Double-click on the “POWER” macro. (The macros are listed at the bottom left corner of the active window.) The “POWER” macro identifies the alleles in the ladder sample and calculates offsets for all of the loci. This process may take several minutes. When completed, a plots window will open to display the allelic ladders (i.e., Penta E, D18S51, D21S11, TH01 and D3S1358).

In general, the allelic ladders contain fragments of the same lengths as either several or all known alleles for the locus. The allelic ladder sizes and repeat units are listed in Table 2 (Section II.B) of this manual. Analysis using GeneScan® Analysis Software and Genotyper® Software allows allele determination by comparison of amplified sample fragments with allelic ladders and internal lane standards. When using an internal lane standard, the calculated lengths of the allelic ladder components may differ from those listed in the table. This is due to differences in migration resulting from sequence differences between the allelic ladder fragments and those of the internal size standard and is not a matter of concern.

6. Double-click on the “Allelic Ladders” macro. A plots window will open to display the blue (fluorescein) dye allelic ladders (i.e., Penta E, D18S51, D21S11, TH01 and D3S1358), the green (JOE) dye allelic ladders (i.e., Penta E, CSF1PO, D16S539, D7S820, D13S317 and D5S818) and the yellow (TMR) dye allelic ladders (i.e., FGA, TPOX, D8S1179, vWA and Amelogenin). Confirm that the correct allele designations were assigned to the allelic ladders (Figure 2).

Note: The software uses one ladder sample for determining the allele sizes. The macro uses the first ladder sample imported for allele designations. If many off-ladder alleles are found in the samples, a different ladder injection or lane can be imported into the macro and the previous ladder cleared. The macro will need to be rerun using the new ladder sample.

7. Double-click on the “Display Fluorescein Data” macro to display the blue dye for all the sample injections/lanes. Scroll down to observe and/or edit as necessary.
8. Double-click on the “Display TMR Data” macro to display the yellow dye for all the sample injections/lanes. Scroll down to observe and/or edit as necessary.
9. Double-click on the “Display JOE Data” macro to display the green dye for all the sample injections/lanes. Scroll down to observe and/or edit as necessary.
10. Create the appropriate table by selecting the “PowerTable,” “Make Allele Table” or “Make CODIS Table” macro. Three table formats are available as macros (see below). The PowerTable option allows up to 4 alleles per sample file. Additional information such as low peak signal or high peak signal is also included. The allele table and CODIS table options include only 2 alleles per locus. If >2 alleles are present at a locus, the smallest alleles identified are included. The allele table format displays the categories (loci) in columns while the CODIS table format displays the categories in rows. You may customize these tables to fit the needs of your laboratory. To save data in tables, go to the “table” dropdown menu and highlight “export” and “table.” The saved file can be viewed and analyzed using Microsoft® Excel.

PowerTable Format

Sample Info	Sample Comment	Category	Peak 1	Peak 2	Peak 3	Peak 4	Over-flow	Low Signal	Saturation	Edited Label	Edited Row

Allele Table Format

Sample Info	Category Allele 1	Category Allele 2						

CODIS Table Format

Sample Info	Category	Peak 1	Peak 2

11. Save the analyzed data, go under “file” and highlight “save as.”

Warning: The PowerTyper™ Macro is a Genotyper® file and can be overwritten if “save” is used instead of “save as.”

C. Controls

1. Observe the results for the negative control. The negative control should be devoid of amplification products.
2. Observe the results for the 9947A DNA positive control. Amplified sample peak heights of less than 2,000rfu are ideal. Compare the control DNA allelic repeat sizes with the locus-specific allelic ladder. The expected 9947A DNA allele designations for each locus are listed in Table 3 (Section II.B).



The PowerTyper™ Macro is a Genotyper® file and can be overwritten if “save” is used instead of “save as”.

D. Results

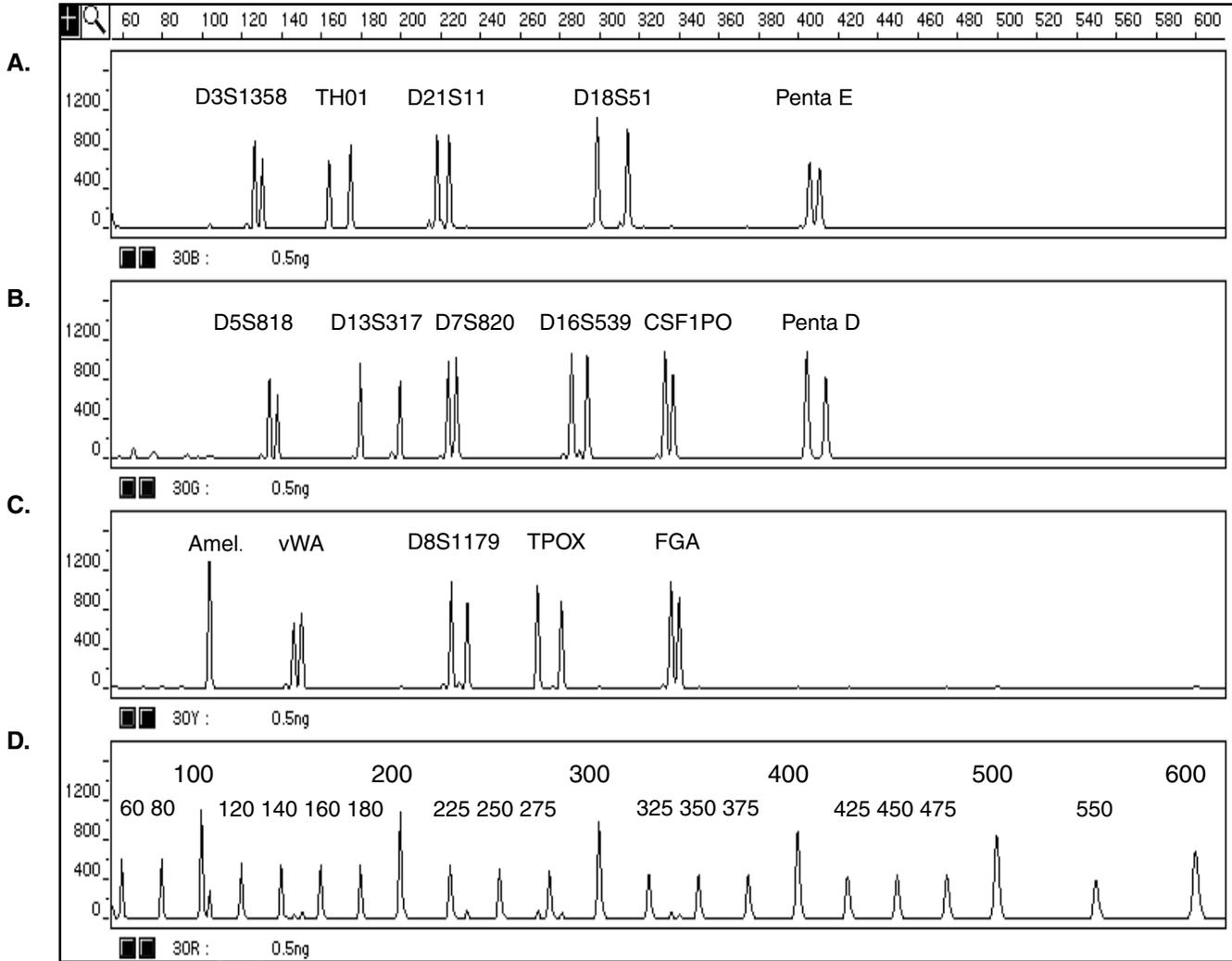


Figure 1. The GenePrint® PowerPlex™ 16 System. A single DNA template (0.5ng) was amplified using the PowerPlex™ 16 10X Primer Pair Mix. The amplification products were mixed with the Internal Lane Standard 600 and run on an ABI PRISM® 310 Genetic Analyzer using a 3-second injection time. The results were analyzed using GeneScan® Analysis Software. **Panel A:** An electropherogram showing the peaks of the fluorescein-labeled loci, D3S1358, TH01, D21S11, D18S51 and Penta E. **Panel B:** An electropherogram showing the peaks of the JOE-labeled loci, D5S818, D13S317, D7S820, D16S539, CSF1PO and Penta D. **Panel C:** An electropherogram showing the peaks of the TMR-labeled loci, Amelogenin, vWA, D8S1179, TPOX and FGA. **Panel D:** An electropherogram showing the fragments of the Internal Lane Standard 600.

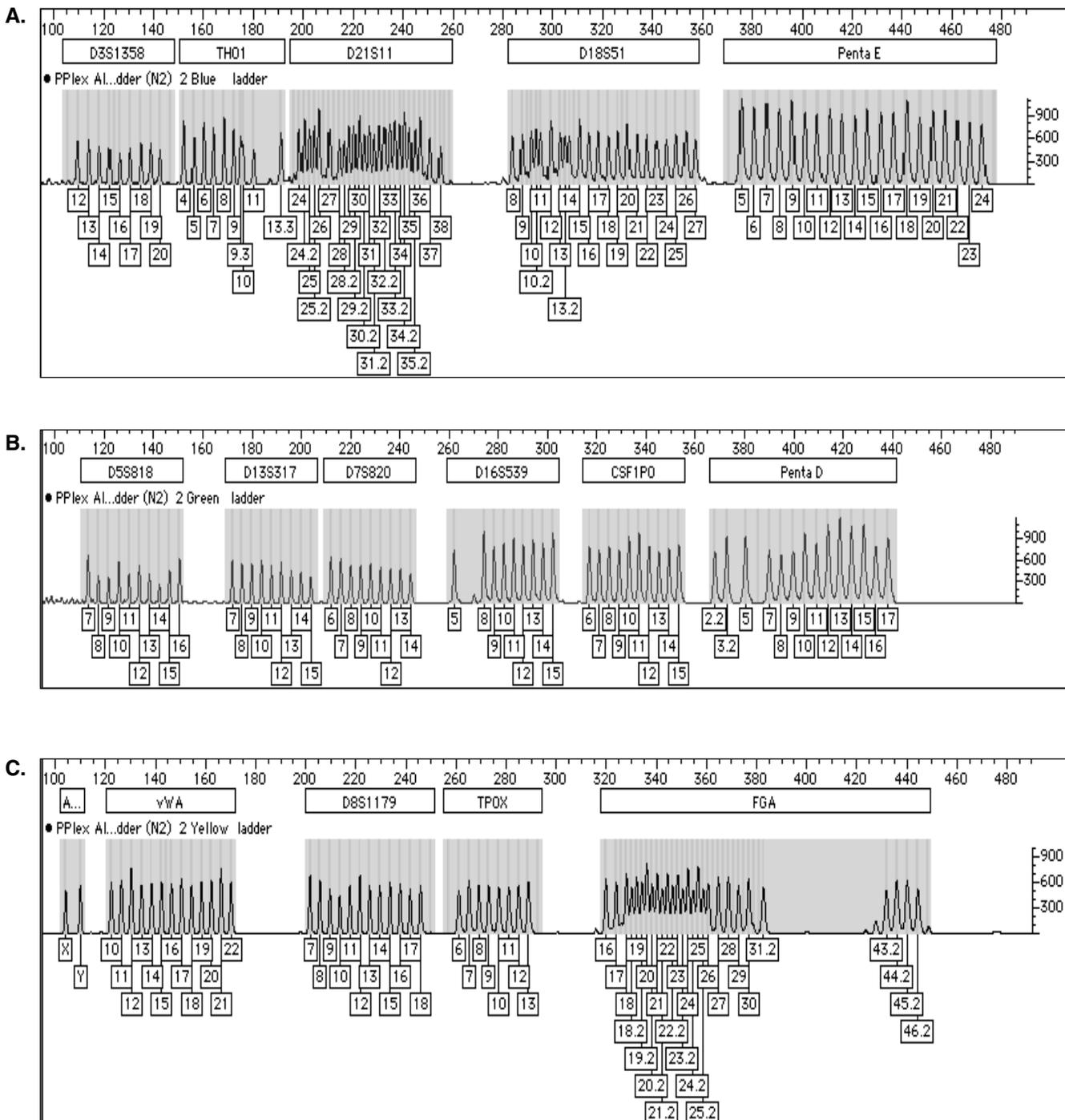


Figure 2. The PowerPlex™ 16 Allelic Ladder Mix. The PowerPlex™ 16 Allelic Ladder Mix was run on an ABI PRISM® 310 Genetic Analyzer using a 3-second injection time. The GeneScan® sample file was analyzed with the Genotyper® 2.0 Software and the PowerTyper™ 16 Macro. **Panel A:** The fluorescein-labeled allelic ladder components and their allele designations. **Panel B:** The JOE-labeled allelic ladder components and their allele designations. **Panel C:** The TMR-labeled allelic ladder components and their allele designations.

IX. Troubleshooting

A. Amplification

Symptoms	Possible Causes	Comments
Faint or no allele peaks	Impure template DNA	Because of the small amount of template used, this is rarely a problem. Depending on the DNA extraction procedure used, inhibitors may be present in the DNA sample.
	Insufficient template DNA	Use the recommended amount of template DNA.
	Insufficient enzyme activity	Use the recommended amount of AmpliTaq Gold® DNA polymerase. Check the expiration date on the tube label.
	Incorrect amplification program	Confirm the amplification program.
	High salt concentration or altered pH	The DNA volume should not be more than 20% of the total reaction volume. Carryover from the DNA sample of K ⁺ , Na ⁺ , Mg ²⁺ or EDTA can have a deleterious effect on PCR. A change in pH may also affect PCR.
	Thermal cycler or tube problems	Review the thermal cycling protocols in Section V.B. We have not tested other reaction tubes or thermal cyclers. Calibration of the thermal cycler heating block may be required.
	Primer concentration too low	Use the recommended primer concentration. Mix well before use.
	Samples not denatured before loading in the gel	Make certain the samples are heat denatured immediately prior to loading the gel or capillary.
	Poor capillary electrophoresis injection (ILS 600 peaks also affected)	Re-inject the sample. Check the syringe O-ring for leakage. Check the laser power.
	Poor quality formamide used	Ensure that high-quality formamide is used when running samples on the ABI PRISM® 310 Genetic Analyzer. The conductivity of the deionized formamide should be <100µS/cm.
Extra peaks visible in one or all of the color channels	Contamination with another template DNA or previously amplified DNA	Cross-contamination can be a problem. Use aerosol-resistant pipette tips and change gloves regularly.

IX. Troubleshooting (continued)

A. Amplification (continued)

Symptoms	Possible Causes	Comments
Extra peaks visible in one or all of the color channels (continued)	Artifacts of STR amplification	PCR amplification of STR systems sometimes generates artifacts that appear as faint peaks, one or four bases below an allele. Stutter band peak heights will be high if the samples are overloaded (i.e., >2000rfu).
	Samples not completely denatured	Heat-denature the samples for the recommended time immediately prior to loading the gel or capillary.
	Background	Load less amplification product or decrease the injection time.
	Capillary electrophoresis (CE) related artifacts ("spikes")	Minor voltage changes or urea crystals passing by the laser may cause "spikes" or unexpected peaks. Spikes sometimes appear in one color but often are easily identified by their presence in more than one color. Re-inject the samples to confirm.
	CE-related artifacts (contaminants)	Contaminants in the water used on the ABI PRISM® 310 Genetic Analyzer and to dilute the 10X Genetic Analyzer buffer may generate peaks in the blue and green dye colors. Use autoclaved water, change vials, and wash buffer reservoir.
	Excessive amount of DNA	1–2ng of DNA template are recommended. Amplification of DNA templates >2ng may result in a higher number of stutter bands. Use less DNA template or reduce the number of cycles in the amplification program by 2–4 cycles (10/20 or 10/18 cycling).
	Pull-up or bleed-through	Pull-up can occur when peak heights are >2,000rfu or if a poor or incorrect matrix has been applied to the samples. Increase the peak amplitude threshold (i.e., 150–200rfu) in the analysis parameters and reanalyze using the GeneScan® Software. Generate a new matrix and apply to the samples.

For questions not addressed here, please contact your local Promega Branch Office or distributor. Contact information available at: www.promega.com.

E-mail: techserv@promega.com

IX. Troubleshooting (continued)

A. Amplification (continued)

Symptoms	Possible Causes	Comments
Allelic ladder is not running the same as the sample	Allelic Ladder and Primer Pair Mix not compatible	Ensure that the Allelic Ladder is from the same kit as the Primer Pair Mix.
	Poor quality formamide used	Ensure that high quality formamide is used when running samples on the ABI PRISM® 310 Genetic Analyzer. The conductivity of the deionized formamide should be <100µS/cm.
	Buffer incompatibility	Samples were diluted in the wrong buffer. Use 1X Gold ST★R Buffer for diluting samples.
	Migration of samples may change slightly over the course of a capillary electrophoresis (CE) run with many samples. This may be due to changes in temperature or in the CE column over time.	Use a different injection of the allelic ladder to determine sizes in the PowerTyper™ 16 Macro.
Imbalance of peak heights	Excessive amount of DNA	1–2ng of DNA template are recommended. Amplification of DNA templates at greater than 2ng results in an imbalance in yields with the smaller loci showing more amplification product than the larger loci. Use less DNA template or reduce the number of cycles in the amplification program by 2–4 cycles (10/20 or 10/18 cycling) to improve locus to locus balance.
	Use of FTA™ paper	Results may be similar to use of excess amounts of DNA template. Reduce the number of cycles in the amplification program by 2–4 cycles (10/20 or 10/18 cycling) to improve locus to locus balance.
	Degraded DNA sample	DNA template is degraded into smaller fragments with the larger loci showing diminished yield.
	Insufficient template DNA	Use the recommended amount of template DNA.
	Poor quality formamide used	Ensure that high quality formamide is used when running samples on the ABI PRISM® 310 Genetic Analyzer. The conductivity of the deionized formamide should be <100µS/cm.

Note: Dilution of over-amplified samples displays dropoff of larger loci.

IX. Troubleshooting (continued)

B. PowerTyper™ 16 Macro

Symptoms	Possible Causes	Comments
File does not open on your computer	Genotyper® Software not installed	Be certain that the Genotyper® 2.0 or 2.5 Software is installed.
	Incorrect version of Genotyper® Software	The PowerTyper™ 16 Macro may not work with versions prior to Genotyper® version 2.0.
	Damaged diskette	The diskette may have been damaged during shipment. Contact Technical Services at 800-356-9526 or 608-274-4330.
Error message: "Could not complete the 'Run Macro' command because no dye/lanes are selected"	Allelic ladder sample files not identified	Be certain the "sample info" column for each lane containing the PowerPlex™ 16 Allelic Ladder Mix contains the word "ladder." The macro uses the word "ladder" to identify the sample files containing allelic ladder.
	All four dye colors not imported	For Genotyper® Software, version 2.0, four dye colors should be checked when importing sample files. For Genotyper® 2.5, set the preferences (under "edit") to import the blue, green, yellow and red colors.
Error message: "Could not complete the 'Run Macro' command because the labeled peak could not be found"	The peak heights for one or more of the alleles in the allelic ladder sample file are below 150rfu	The allelic ladder categories are defined as having a minimum peak height of 150rfu. If the ladder alleles are below 150rfu then the software will not be able to locate the allele peak. Rerun the allelic ladder using more sample or longer injection time to assure peak heights above 150rfu.
	CE spikes in the allelic ladder sample are being identified as alleles by the macro	Use a different injection of the allelic ladder.
	Occasionally the TH01 alleles 9.3/10 do not separate when using heavy smoothing in the GeneScan® analysis parameters	Use light smoothing in the GeneScan® analysis parameters.
	Allelic ladder peaks are too high causing stutter peaks to be called as allele peaks	Use a shorter injection time, decrease the amount of allelic ladder used, or reanalyze the allelic ladder sample using increased peak amplitude thresholds in the GeneScan® analysis parameters.

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IX. Troubleshooting (continued)

B. PowerTyper™ 16 Macro (continued)

Symptoms	Possible Causes	Comments
Error message: “Could not complete the ‘Run Macro’ command because the labeled peak could not be found” (continued)	The base pair size of the allelic ladder alleles does not fall within the defined category range	Confirm that the internal lane standard fragments are correct. Redefine the internal lane standard fragments and reanalyze the sample using GeneScan® Software
		Compare the size of the smallest allele in the allelic ladder with the base pair size and range listed in the categories for the same alleles. If necessary, increase the category start range (in the category window) to greater than ±6bp and save the macro under a new name.
The plots window or allele table does not display all data	The macros were not run in the proper order	The “ POWER ” macro must be run first before the remaining macros are run.
Many off-ladder peaks in the samples using the ABI PRISM® 310 Genetic Analyzer.	Migration of samples may change slightly over the course of CE run with many samples. This may be due to changes in temperature or in the CE column over time.	Use a different injection of the allelic ladder to determine sizes in the PowerTyper™ 16 Macro. Do not use the first injection on a new column for the ladder sample.
	The base pair size of the alleles is incorrect due to incorrect fragment sizes assigned to the internal lane standard	Confirm that the internal lane standard fragments are assigned correctly. Reanalyze the sample using GeneScan® Software and redefine the internal lane standard fragments.

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B. Additional STR References

The fields of forensic and paternity analysis are changing rapidly. For this reason, it is difficult to keep our manuals up-to-date regarding new technologies and new publications. However, a substantial reference list of publications describing STRs and much related information can be found at an Internet web site created by the National Institutes of Science and Technology (NIST) Biotechnology Division (<http://www.cstl.nist.gov/biotech/strbase/>) This web site is occasionally updated and has numerous links to many other useful sites.

The articles below provide an overview of the listed topics and can be found on the Promega web site, <http://www.promega.com/geneticidentity/>. The spreadsheet for the PowerStats application is also available from this site. If you cannot access the web site, please contact your Promega Branch Office or distributor.

- 1. The Short Tandem Repeat DNA Database Web Site:**
Butler, J.M. and Reeder, D.J. (1997) STRBase: A short tandem repeat DNA database. *Profiles in DNA* **1**(2), 10. (http://www.promega.com/profiles/102/102_10/)
- 2. Previously released Promega STR products:**
Schumm, J.W. (1997) *GenePrint*® STR multiplexes: reliability, flexibility, and throughput in database and casework-compatible STR analysis. *Profiles in DNA* **1**(1), 9. (http://www.promega.com/profiles/101/101_09/)
- 3. Use of the *GenePrint*® PowerPlex™ 1.2 System Using Capillary Electrophoresis:**

Demers, D.B., Kelly, C.M. and Sozer, A.C. (1998) Multiplex STR analysis by capillary electrophoresis. *Profiles in DNA* **1(3)**, 3. (http://www.promega.com/profiles/103/103_03/)

4. Pentanucleotide Repeat Loci:

Bacher, J. and Schumm, J.W. (1998) Development of highly polymorphic pentanucleotide tandem repeat loci with low stutter. *Profiles in DNA* **2(2)**, 3. (http://www.promega.com/profiles/202/202_03/)

5. PowerStats Analysis of Population Data:

Tereba, A. (1999) Tools for analysis of population statistics. *Profiles in DNA* **2(3)**, 14. (http://www.promega.com/profiles/203/203_14/)

XI. Appendix

A. Composition of Buffers and Solutions

10% ammonium persulfate

Add 0.05g of ammonium persulfate to 500µl of deionized water. Use 250µl of 10% ammonium persulfate for each 50ml of acrylamide gel solution.

Blue Dextran Loading Solution

88.25% Formamide
15mg/ml Blue Dextran
4.1mM EDTA (pH 8.0)

deionized formamide

Use the Ultra Pure Grade from Amresco (Cat.# 0606). Use a conductivity meter to check the conductivity. The deionized formamide must have a conductivity of less than 100µS/cm. Deionized formamide can be aliquoted and frozen. Avoid multiple freeze-thaws.

ethidium bromide solution (10mg/ml)

1.0g ethidium bromide

Dissolve the ethidium bromide in 100ml of deionized water. Wrap in aluminum foil or transfer the solution to a dark bottle and store at room temperature. **Caution:** Ethidium bromide is a powerful mutagen. Gloves should be worn when working with the dye, and a mask should be worn when weighing it out.

Gold ST★R 10X Buffer

500mM KCl
100mM Tris-HCl (pH 8.3 at 25°C)
15mM MgCl₂
1% Triton® X-100
2mM each dNTP
1.6mg/ml BSA

TAE 50X buffer (pH 7.2)

242g Tris base
57.1ml glacial acetic acid
100ml 0.5M EDTA stock

Add the Tris base and EDTA stock to 500ml of deionized water. Add the glacial acetic acid. Bring to 1 liter with deionized water.

TBE 10X buffer

107.8g Tris base
7.44g EDTA
(Na₂EDTA • 2H₂O)
~55.0g boric acid

Dissolve the Tris base and EDTA in 800ml deionized water. Slowly add the boric acid and monitor the pH until the desired pH of 8.3 is obtained. Bring the volume to 1 liter with deionized water.



**TE-4 [10mM Tris-HCl, 0.1mM EDTA
(pH 7.5)]**

2.21g Tris base
0.037g EDTA
(Na₂EDTA • 2H₂O)

Dissolve Tris base and EDTA in 900ml deionized water. Adjust to pH 7.5 with HCl. Increase volume to 1 liter with deionized water.

B. Related Products

GenePrint® Fluorescent STR Multiplex System

Product	Size	Cat.#
<i>GenePrint</i> ® PowerPlex™ 1.2 System	100 reactions	DC6101
	400 reactions	DC6100

GenePrint® Sex Identification Systems

Product	Size	Cat.#
<i>GenePrint</i> ® Fluorescent Sex Identification System-Amelogenin (Fluorescein)	100 reactions	DC5171
	400 reactions	DC5170
<i>GenePrint</i> ® Fluorescent Sex Identification System-Amelogenin (TMR)	100 reactions	DC6171
	400 reactions	DC6170

Accessory Components

Product	Size	Cat.#
Internal Lane Standard 600	150µl	DG2611
Fluorescent Ladder CXR, 60-400 Bases	65µl	DG6221
<i>GenePrint</i> ® Matrix FL-JOE-TMR-CXR	20µl	DG2860
	(each dye)	
Gold ST★R 10X Buffer	1.2ml	DM2411
Mineral Oil	12ml	DY1151
Nuclease-Free Water	50ml	P1193
	(2 × 25ml)	

DNA Purification Systems

Product	Size	Cat.#
ReadyAmp™ Genomic DNA Purification System	100 isolations	A7710
Wizard® Genomic DNA Purification Kit	100 isolations	A1120
	500 isolations	A1125
SV Total RNA Isolation System	50 preps	Z3100

B. Related Products (continued)
Polyacrylamide Gel Electrophoresis Reagents

Product	Size	Cat.#
Ammonium Persulfate	25g	V3131
TBE 10X Solution	1L	V4251
TEMED	50ml	V3161
Urea	1kg	V3171

ART® Aerosol-Resistant Tips (See Table 11)

Product	Volume	Size (tips/pack)	Cat.#
ART® 10 Ultramicro Pipet Tip	0.5–10µl	960	DY1051
ART® 10F Pipet Tip	0.5–10µl	960	DY1141
ART® 20E Ultramicro Pipet Tip	0.5–20µl	1,000	DY1061
ART® 20P Pipet Tip	20µl	960	DY1071
ART® GEL Gel Loading Pipet Tip	100µl	1,000	DY1081
ART® XL Xtra Long Pipet Tip	100µl	800	DY1091
ART® 100 Pipet Tip	100µl	960	DY1101
ART® 100E Pipet Tip	100µl	960	DY1111
ART® 200 Pipet Tip	200µl	960	DY1121
ART® 1000 Pipet Tip	1,000µl	768	DY1131



Table 11. Cross-Reference Table for Pipettes Compatible with the ART® Aerosol-Resistant Tips.

Pipettors	ART® 10 DY1051	ART® 10F DY1141	ART® 20E DY1061	ART® 20P DY1071	ART® GEL DY1081	ART® XL DY1091	ART® 100 DY1101	ART® 100E DY1111	ART® 200 DY1121	ART® 1000 DY1131
Eppendorf® Digital, 0.5–10µl			•							
Eppendorf® Digital, 2–10µl, 2–20µl				•				•		
Eppendorf® Digital, 10–100µl					•	•				
Eppendorf® Digital, 100–1000µl										•
Finnpipette® Digital, 0.5–10µl		•								
Finnpipette® Digital, 5–40µl				•	•		•	•		
Finnpipette® Digital, 40–200µl					•				•	
Finnpipette® Digital, 200–1000µl										•
Oxford Benchmate®, 0.5–10µl	•		•							
Oxford Benchmate®, 10–50µl							•			
Oxford Benchmate®, 40–200µl					•	•		•	•	
Oxford Benchmate®, 200–1,000µl										•
Oxford Benchmate® Multi-channel, 5–50µl							•			
Oxford Benchmate® Multi-channel, 40–200µl									•	
Pipetman® P-2, 0–2µl	•									
Pipetman® P-10, 0.5–10µl	•									
Pipetman® P-20, 0–20µl				•						
Pipetman® P-100, 0–100µl					•	•		•		
Pipetman® P-200, 0–200µl									•	
Pipetman® P-1000, 0–1,000µl										•
Titertek®/Labsystems Multi-channel, 5–50µl							•			
Titertek®/Labsystems Multi-channel, 50–300µl									•	
Ulster V-3 Series, 2–20µl				•						
Ulster V-3 Series, 20–200µl						•			•	
Ulster V-3 Series, 200–1,000µl										•

^(a)STR loci are the subject of U.S. Pat. No. 5,766,847 and German Pat. No. DE 38 34 636 C2, issued to the Max-Planck-Gesellschaft zur Förderung der Wissenschaften, eV, Germany. Exclusive rights have been licensed to Promega Corporation for uses in human clinical research and diagnostics applications and all forms of human genetic identity. The development and use of STR loci is covered by U.S. Pat. No. 5,364,759 assigned to Baylor College of Medicine, Houston, Texas. Rights have been licensed to Promega Corporation for all applications.

U.S. Pat. No. 5,599,666 has been issued to Promega Corporation for allelic ladders for the loci CSF1PO, F13A01, FESFPS, LPL and vWA. U.S. Pat. No. 5,674,686 has been issued to Promega Corporation for allelic ladders for the locus CSF1PO and the combination of allelic ladders for the loci CSF1PO, FESFPS and TH01. U.S. Pat. No. 5,783,406 has been issued to Promega Corporation for allelic ladders for the locus CSF1PO.

Use of the *GenePrint*[®] STR Systems requires performance of the polymerase chain reaction (PCR), which is the subject of European Pat. Nos. 201,184 and 200,362, and U.S. Pat. Nos. 4,683,195, 4,965,188 and 4,683,202 owned by Hoffmann-La Roche. Purchase of the *GenePrint*[®] STR Systems does not include or provide a license with respect to these patents or any other PCR-related patent owned by Hoffmann-La Roche or others. Users of the *GenePrint*[®] STR Systems may, therefore, be required to obtain a patent license, depending on the country in which the system is used. For more specific information on obtaining a PCR license, please contact Hoffmann-La Roche.

^(b)U.S. Pat. No. 5,843,660 has been issued to Promega Corporation for multiplex amplification of STR loci.

^(c)Patent Pending.

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